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Effect of *Adhatoda vasica* (L.) Nees Leaf Extract Prepared by two Different Methods on Mitosis of Root Meristematic Cells of *Allium cepa* L.

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Abstract: *Adhatoda vasica* is a well-known plant drug in Ayurvedic and Unani medicine. *Adhatoda* leaves have been used extensively in Ayurvedic Medicine primarily for respiratory disorders. Aqueous leaf extract of *A. vasica* was used to investigate its cytotoxic and genotoxic effects on root meristematic cells of *Allium cepa*. 1, 2.5, 5, 10 and 20% concentrations of leaf extract prepared by two different ways were used in the present study. It was found that leaf extract of *A. vasica* exhibits mitodepressive activity as well as it induces disorder of chromosome kinetic including formation of sticky chromosome, c-metaphase, metaphasic and anaphasic disorders in *Allium cepa* root meristem.

Keywords: *Adhatoda vasica*, Leaf Extract, *Allium cepa*, Genotoxicity, Cytotoxicity.

1. Introduction

Adhatoda vasica is a well-known plant drug in Ayurvedic and Unani medicine (Manjunath, 1948). The medicinal properties of *Adhatoda vasica* called Vasa or Vasaka in Sanskrit. The plant has been used in the indigenous system of medicine in India for over 2000 years (Atal, 1980). *Adhatoda* leaves have been used extensively in Ayurvedic Medicine primarily for respiratory disorders including cough, cold, asthma, bronchitis etc. An important chemical constituent of leaf includes pyrroloquinazoline alkaloids, vasicine, vasicol, adhatonine, vasicinone, vasicinol and vasicinolone (*Ref. 13*). The roots are known to contain vasicinolone, vasicol, peganine and 2'-hydroxy-4-glucosyl-oxychalcone. The flowers contain b-sitosterol-D-glucoside, kaempferol, its glycosides and quereetin. Vasicine has been considered as active principle of *A. vasica* which shows numerous pharmacological activities viz., antimalarial (Chopra, 1955; Bose, 1932; Agharkar, 1953), anti-inflammatory (Chakraborty and Brantner 2001; Srinivasrao *et al*., 2006), antioxidant (Srinivasrao *et al*., 2006; Padmaja *et al*., 2011), antidiabetic (Clamp *et al*., 1979; Gao *et al*., 2008), antibacterial (Ilango *et al*., 2009) etc. *A. vasica* leaves have been used in the treatment of diarrhoea, dysentery, tuberculosis, skin diseases, vomiting and leprosy etc. Malla *et al*., 1982 have reported that *A. vasica* leaves have been consumed as vegetable in Nepal and India. Review of literature shows that there is no evidence or indication of any serious adverse effect except abortifacient effect (Nath *et al*., 1997) of *A. vasica* extract.

Classical method for extracting the juice (*swarasa*) from the leaf is an elaborate process, which involves subjecting a bolus of crushed fresh leaf to heat followed by squeezing out the juice. Commercially, to prepare the juice of *Vasaka*, manufacturers have been adopting different methods other than the traditional method.

All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy- Paracelsus (Darrel Crain, 2007). Thus the present study intends to evaluate the effect of *A. vasica* extract prepared by two different methods on mitosis of root meristematic cells of *Allium cepa*.

2. Materials and Methods

2.1 Collection of plant material

The leaves of *A. vasica* were collected from the Medicinal Plant Garden of Life Sciences Department, Dibrugarh University.

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2.2 Preparation of leaf extract

A. vasica leaf extract was prepared by two methods-

a) Fresh leaves of *A. vasica* were harvested and thoroughly washed in tap water. 100gm of leaves were macerated to paste with the help of sterilized mortar and pestle with 100ml tap water and it was filtered through muslin cloth. The filtrate was kept frozen at 4°C and used in subsequent experiments as stock solution and coded as LE-1.

b) 100g of fresh leaves were crushed using mortar and pestle and placed in a steel vessel without adding any water and heated at 121°C (15 lb pressure) for 30 mins. The crushed leaves were taken in 4 layers of muslin cloth and squeezed to obtain leaf juice (Soni *et al.*, 2008). The extracted juice was kept frozen at 4°C and considered as stock solution in subsequent experiments and coded as LE-2.

2.3 *Allium cepa* test

Onion bulbs were commercially obtained from new market, Dibrugarh. Before use, the loose outer scales were carefully removed and dry bottom plates were scraped away without destroying the root primordia.

Five concentrations (v/v) of the extract were prepared from the stock, viz: 1, 2.5, 5, 10 and 20% to study mitotic and genotoxic effects. Tap water was used as control. For each concentration, five onion bulbs were set up and allowed to produce root in tap water for 24 hrs. On next day the bulbs were transferred to test samples as well as control. Roots were treated in the aforesaid test samples for 48 hrs. After end of 48 hrs, the length of the roots were measured with a ruler and other morphological abnormalities were recorded. To study the genotoxic effect, after the end of 48 hrs of treatment with the aforesaid concentrations, root tips from each concentration were fixed in aceto-alcohol (1:3) fixative for 24 hrs. After 24 hrs, root tips were transferred to 70% ethyl alcohol and stored at 4°C.

For chromosomal analysis, root tips were hydrolyzed in 1N HCl and 2% aceto-carmine (1:9) for 1-2 mins and kept for overnight. On next day root tips were squashed in 1% aceto-carmine as proposed by Sharma and Sharma, 1983 and the coverslips were sealed on the slides with clear fingernail polish as suggested by Grant, 1982. Five slides were prepared for each treatment and control and 1000 cells were scored per slide to study the mitotic index and aberrant cells. The slides were analyzed at x 1000 magnification. The Mitotic Index (MI) was calculated as the number of dividing cells per total cells scored at each concentration. The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored for each concentration of the extract (Bakare *et al.*, 2000). The mitotic inhibition was obtained as:

$$\text{Mitotic inhibition} = \frac{(\text{MI in control} - \text{MI in treated group})}{\text{MI in control}} \times 100$$

2.4 Statistical analysis

The SPSS 15.0 statistical package was used for the analysis. The difference between the control and the treated groups in relation to root length and root number was analyzed by Students' t-test.

3. Results and Discussion

The present investigation showed that all the tested concentrations of aqueous leaf extract (LE-1 and LE-2) of *A. vasica* inhibit significant root growth in comparison to control. Inhibition of root length and root number was greater with increasing concentration of leaf extracts (Table 1 and Table 2). Root length in 1% concentration was found nearly equal to the control. Normal root morphology was recorded in all the used concentrations and control. The roots treated in 20% concentrations appeared slightly brown in colour.

Table 1. Morphological and cytological effects of aqueous leaf extract (LE-1) of *A. vasica* on *Allium cepa* root tip cells.

Concentration (%)	Control	1	2.5	5	10	20
Av. of root length (cm) ± S.E.	5.75* ± 0.05	4.86* ± 0.07	4.5* ± 0.08	3.56* ± 0.07	2.31* ± 0.06	1.35* ± 0.07
Av. No. of root ± S.E.	27.2* ± 1.03	22.8* ± 0.52	14.6* ±1.04	9.6* ± 0.73	6.4* ± 0.46	3.4* ± 0.46
No. of dividing cell	298	243	216	177	109	82
Mitotic Index	5.96	4.86	4.32	3.54	2.18	1.64
Mitotic inhibition	0.0	18.46	27.52	40.60	63.42	72.48
Aberrant cell (%)	0.0	3.32	3.02	3.22	0.94	0.64

*Significant difference between control and treated groups using Students' t-test at 95% confidence limit with 9 degrees of freedom.

Table 2. Morphological and cytological effects of aqueous leaf extract (LE-2) of *A. vasica* on *Allium cepa* root tip cells.

Concentration (%)	Control	1	2.5	5	10	20
Av. of root length (cm) ± S.E.	5.94* ± 0.05	5.06* ± 0.08	4.66* ± 0.08	2.3* ± 0.08	1.27* ± 0.07	1.03* ± 0.07
Av. No. of root ± S.E.	28.8* ± 1.03	22.2* ± 0.66	16.4* ± 0.83	10.0* ± 0.63	5.8* ± 0.33	2.8* ± 0.33
No. of dividing cell	291	216	154	0.0	0.0	0.0
Mitotic Index	5.82	4.32	3.08	0.0	0.0	0.0
Mitotic inhibition	0.0	25.77	47.08	100	100	100
Aberrant cell (%)	0.0	0.86	0.72	0.0	0.0	0.0

*Significant difference between control and treated groups using Students' t-test at 95% confidence limit with 9 degrees of freedom.

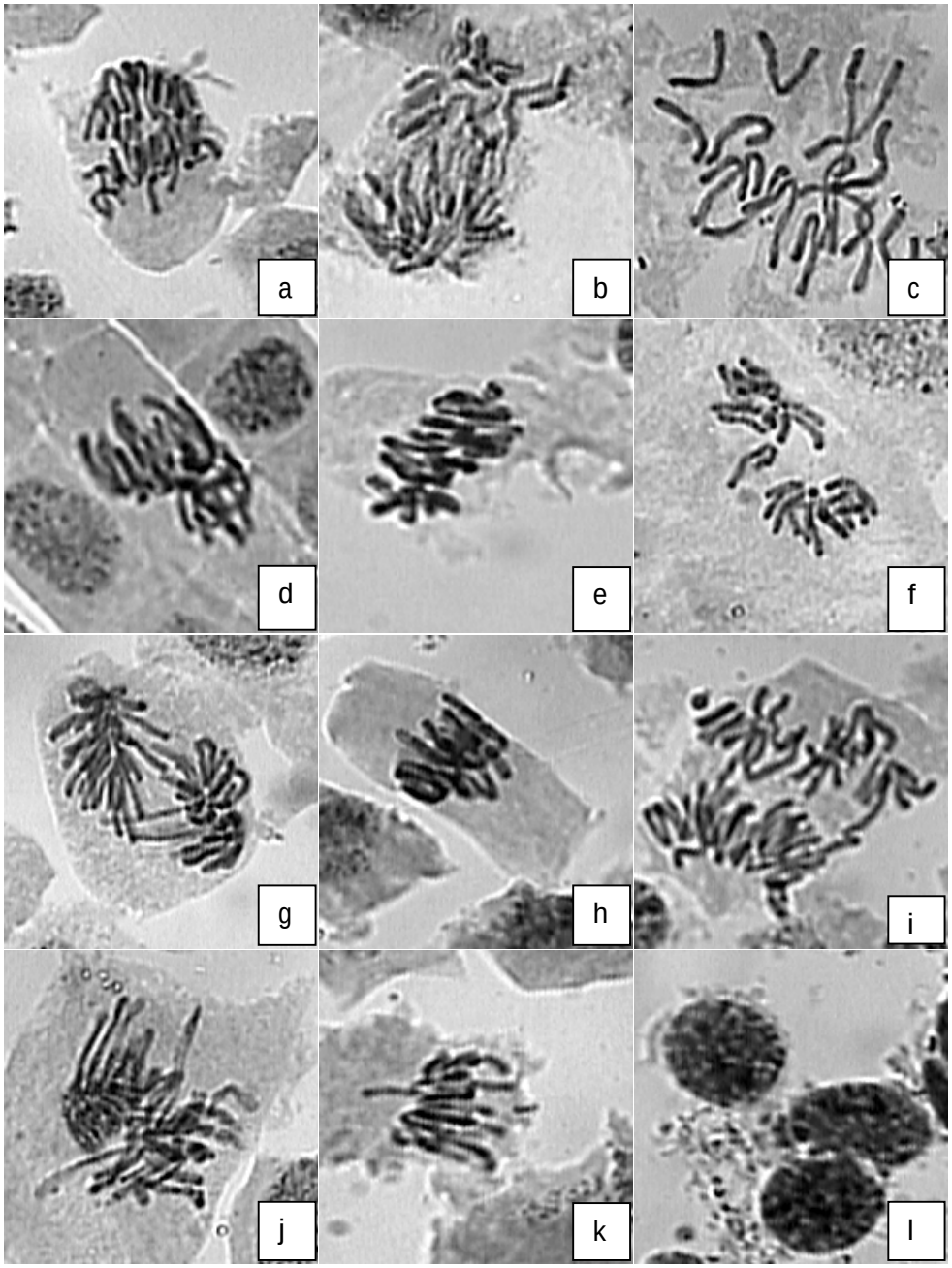

Plate 1. Effect of LE-1 (a-i) and LE-2 (j-l) on mitosis of *Allium cepa* root. a) and b) Disordered anaphase in 1% concentration, c) C-metaphase in 1% concentration, d) Disordered metaphase in 2.5% concentration, e) Sticky metaphase in 2.5% concentration, f) and g) Disordered anaphase in 5% and 10% concentrations respectively, h) Sticky metaphase in 20% concentration, i) Disordered anaphase in 20% concentration, j) Disorder of chromosome kinetic in 1% concentration, k) Unidirectional movement of anaphasic chromosomes in 2.5% concentration and l) Non dividing cells (interphase) in 5% concentration.

While studying the effect of LE-1 of *A. vasica* on MI, dividing cell was noticed in all the treated concentrations and in case of LE-2, no dividing cell was recorded in 5, 10 and 20% concentrations (Table 2). This proves that leaf extract of *A. vasica* interferes with normal sequences of mitotic cell cycle in an inhibiting manner. It was also noticed that there were concentration dependent decrease of MI in concentrations 1, 2.5, 5, 10, 20% and 1, 2.5% as compared to the control in case of LE-1 and LE-2 respectively. Reasons of reduction of mitotic activity might be due to blockade of G2 phases of cellular cycle, inhibition of DNA/protein synthesis etc., (Scheiderman *et al.*, 1971 and Turkoglu, 2007). Mitodepressive effects of some plant extracts resulting from their interaction with DNA nucleotides thus inhibiting DNA synthesis and subsequent mitotic inhibition have been reported by Mercykutly and Stephen, 1980, Schulze and Kirschner, 1996 and Soliman, 2001.

An analysis of chromosomal aberration showed that LE-1 of *A. vasica* causes metaphasic and anaphasic disorders (Fig. 1), sticky chromosome, C-metaphase. These anaphase and metaphasic disorders are indicative of disrupted kinetic of chromosomes and are generated due to qualitative and quantitative changes of chromatin kinetochore (Schneiderman *et al.*, 1971 and Amin, 2002), therefore may indirectly constitute a risk of aneuploidy (Maluszynska and Juchimiuk, 2005). Presence of c-metaphase indicates (PLATE-1.c) that LE-1 also interferes with spindle fibre formation. It was observed that induction of mitotic abnormalities is not concentration dependent. While LE-2 in concentrations 1 and 2.5% showed a few metaphasic and anaphasic disorders (Fig. 2). The present study reveals that the extract of the plant prepared by different ways works in different efficacies. Thus it is a matter of anxiety that if *A. vasica* leaf extract can cause mitotic depression and disorder of chromosome kinetic in plant system than it may also cause the same abnormalities in animal system too particularly in human beings.

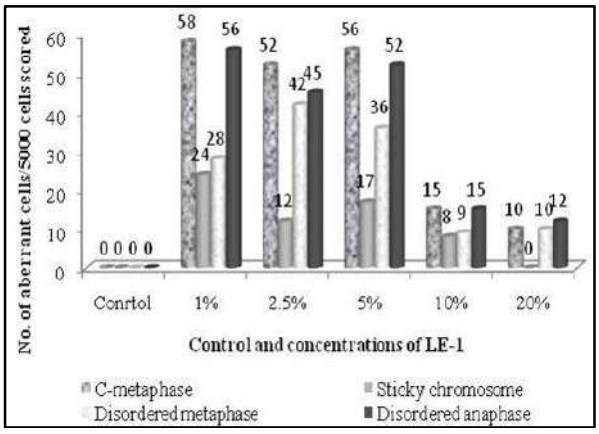

Fig. 1. Number of aberrant cells induced by different concentrations of aqueous leaf extract (LE-1) of *A. vasica* on *Allium cepa* root meristematic cells.

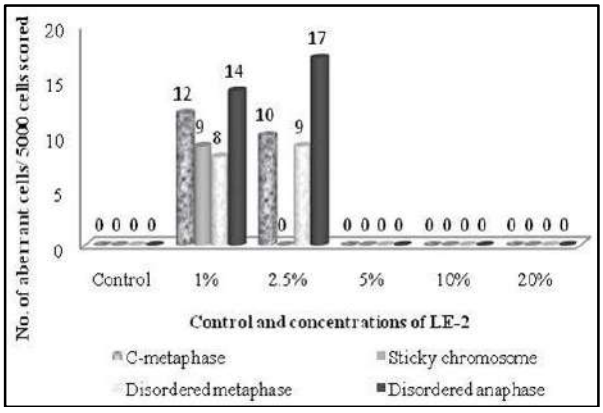

Fig. 2. Number of aberrant cells induced by different concentrations of aqueous leaf extract (LE-2) of *A. vasica* on *Allium cepa* root meristematic cells.

4. Conclusion

Finally, it can be concluded that extracts of *A. vasica* prepared by different methods can cause mitotic depression as well as disorder of chromosome kinetic leading to development of sticky chromosome, c-metaphase and metaphasic and anaphasic disorders. The results of this study suggest that, although *A. vasica* has beneficial effects as a medicinal herb, it can cause problems and damage on cells if not consumed in proper dose and for proper period. Moreover, there is a need for a closer look at the genotoxicological effects of the tested extracts in animal test systems for human welfare as *A. vasica* has been consumed to cure varieties of diseases and as vegetable.

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